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OCCURRENCE OF 5-HYDROXYMETHYLFURFURAL IN VACUUM FOAM-DRIED WHOLE MILK AND ITS RELATION TO PROCESSING AND STORAGE

J. C. CRAIG, JR., N. C. ACETO, AND E. S. DELLA MONICA
Eastern Regional Research Laboratory,[1] Philadelphia, Pennsylvania

SUMMARY

The relationship of 5-hydroxymethylfurfural (HMF) and its precursors to flavor storage stability was investigated. Vacuum foam–dried whole milk was produced and stored at 73° F. HMF values were determined initially, and were found to range from ~1-5 μM/liter. Flavor stability, as a function of storage time, was determined by comparison to freshly dried powder in a Rank Paired Comparison test. A significant relationship was found between flavor stability and the initial HMF value.

Formation of HMF during drying was studied as a function of maximum drying temperature. Twenty-five runs were made, wherein the maximum foam temperature was varied from 103 to 200° F. An Arrhenius-type equation was found to correlate rate of formation of HMF with maximum drying temperature. Analysis of variance showed this relationship to be highly significant.

The flavor of dry whole milk during processing and storage is subject to degradation by many means, for the most part poorly understood. Patton, in a review article, gives Maillard browning of lactose in milk as one of these pathways (4). He also has shown that hydroxymethylfurfural (HMF) is produced by heating lactose with casein (3). Hodge reviews a scheme whereby the Amadori rearrangement can produce HMF and its precursors (1). Keeney and Bassette, in a study of HMF in instantized and noninstantized nonfat dry milk, developed a digestion method which detects total HMF [free HMF and its precursors (2)]. They found that high values of HMF by this test were coincident with off-flavors, particularly cereal, in the skim powders. They suggest determination of HMF as a means of detecting early stages of the browning reaction.

It is the purpose of this paper to show a relationship between HMF, as measured by Keeney and Bassette's analysis, and the storage life of dry whole milk. It is further intended to relate this analysis to process conditions during drying of the whole milk and thence to storage stability.

EXPERIMENTAL PROCEDURES

Production of dry whole milk. Dry whole milk was produced by the method of Sinnamon *et al.* (5). Fluid whole milk is concentrated to 45-48% solids in a falling-film, recirculation type, vacuum evaporator, homogenized in one pass at 3,000 p.s.i. and for 1 to 1½ min. at 500 p.s.i. while metering in finely dispersed N_2. The gassed concentrate is chilled, flowed into trays, and placed in

Received for publication May 29, 1961.

[1] Eastern Utilization Research and Development Division, Agricultural Research Service, USDA.

a vacuum shelf dryer. A vacuum is applied, resulting in a rigid milk foam structure, which is then dried. Temperature control is achieved by following the foam-tray boundary temperature with a thermocouple at that point. Normal production runs are made at a boundary temperature which reaches a maximum and a plateau at $\sim 110°$ F. Drying time ranges from 1½ to 4 hr. The time is a function of foam height, concentrate solids, and terminal moisture.

Determination of HMF. HMF was determined by the Method B of Keeney and Bassette (2), with some modification. It was found necessary to cool the test tube tops to insure complete condensation of the water vapor. This was accomplished by constructing a water bath having a metal plate cut to serve as a test tube holder and a steam barrier. The height of the shield was set so that the digestion mixture in the test tube was submerged and just below the metal shield. The portion of the test tube above the shield was cooled by directing a fan across the bath. Powders were reconstituted to a total solids equivalent to that in the starting milk, i.e., 12%. Digestion was begun within 5 min. of reconstitution. The method yields HMF values with the absorbance of raw milk as a reference point (equal to 0).

References made hereafter to HMF refer to the results of this analysis.

Organoleptic analysis. A typical test used in our storage stability studies is designed to show the effect of some processing or packaging treatment. Thus, two samples, a control and a treated variable, are stored. Organoleptic analysis is by the Rank Paired Comparison Test (RPC), as described by Terry, Bradley, and Davis (6). In this test three or more samples are compared by presenting them in all combinations as pairs to a taste panel; the pairs are presented separately. Even though there are three or more samples tested, valid comparisons between any two can be made. In our studies, three samples are tested; a freshly dried control, a stored control, and a stored variable. The data used in this study represent only a comparison between the stored control and the fresh control. It is not within the scope of this paper to treat the effect of the process or packaging variables.

The taste panel for these tests numbers 15 to 20 judges, who were previously screened and trained for taste acuity. The criterion is, "Which sample of each pair tastes more like fresh milk?" Statistical analysis is by analysis of variance. Results of the RPC tests are expressed as fresh control is fresher than the stored sample at X% significant level of difference (SLD). SLD = 100% represents no difference in the two samples, whereas SLD = 0.1% represents a marked difference.

Storage studies. Powder was dried in the manner described above to a moisture content of $\sim$ 2-3%. It initially had consistent good flavor and physical properties. The powder was analyzed for HMF, packaged in N_2, 0.1 to 0.5% O_2 in the can's headspace, and stored at 73° F.

Samples of the stored powder were withdrawn at intervals and tested organoleptically.

Drying temperature studies. Whole milk powder was produced in the manner described, except that maximum foam temperature, T_m, (normally that at the

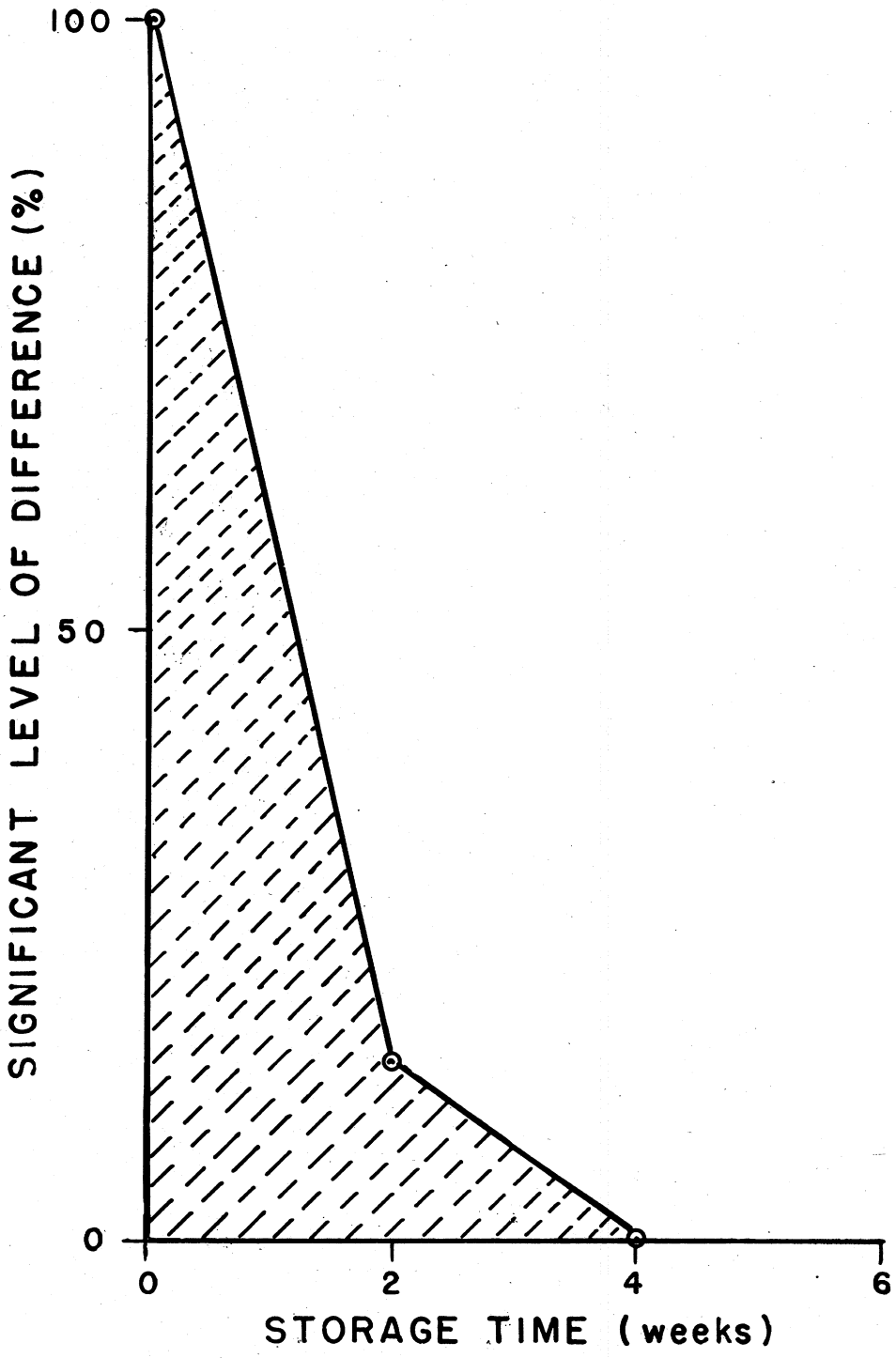

FIG. 1. Example of data from rank paired comparison test as significant level of difference between stored sample and fresh control vs. storage time. Shaded area represents Storage Stability Index (SSI).

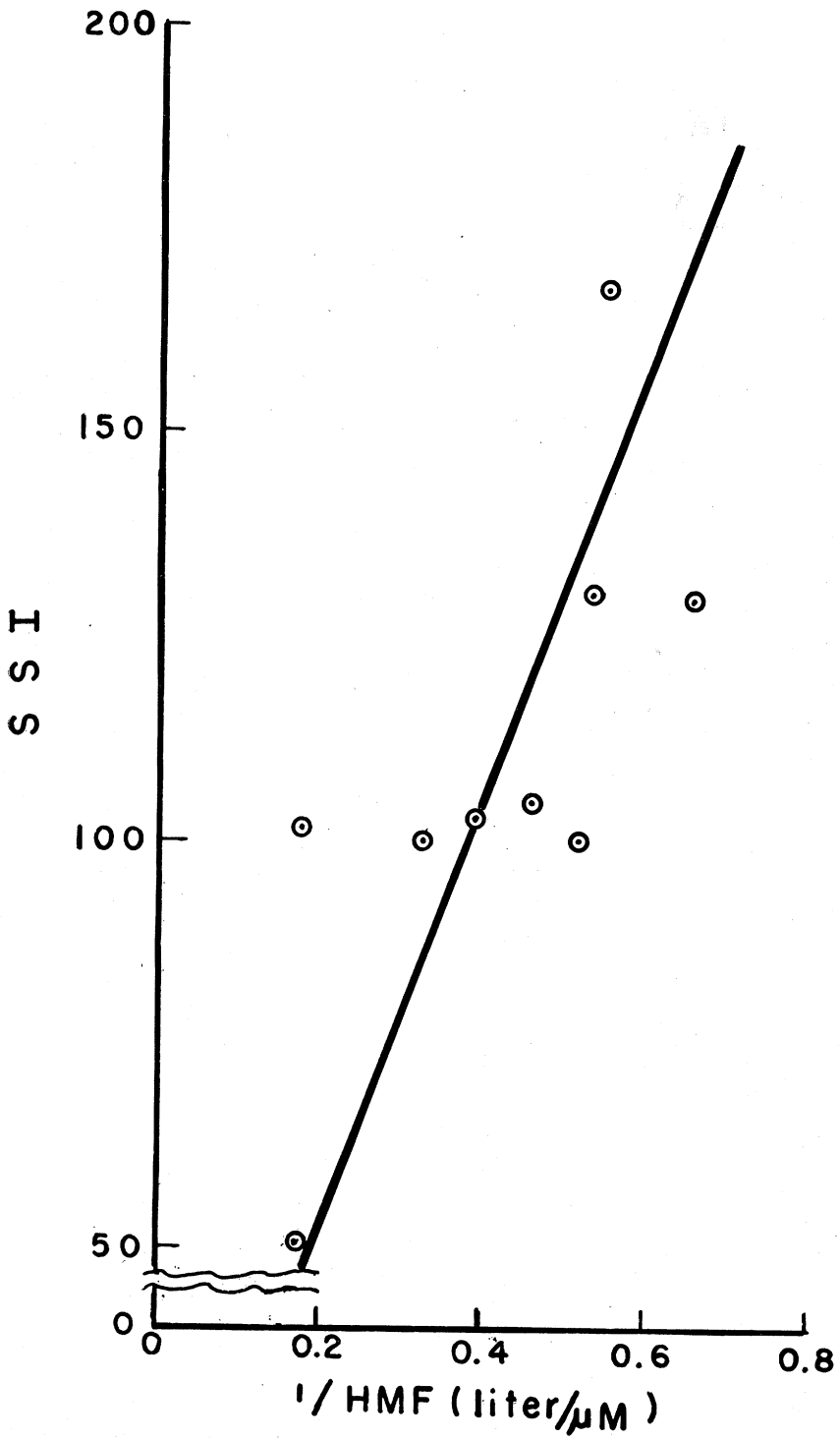

FIG. 2. Storage Stability Index (SLD × storage time) vs. $\frac{1}{\text{HMF}}$ for stored controls at zero time.

foam-tray boundary) was varied. T_m ranged from 103 to 200° F. HMF analyses were made on the freshly dried samples. These runs were made during January-March, 1959, and January-February, 1961.

RESULTS

Storage. Nine storage tests were made. For each, results of the periodic RPC tests were plotted as per cent Significant Level of Difference between freshly dried control and stored control vs. time. An example of this is shown in Figure 1. To quantitatively express the relative storage stability of these powders, the area included in this plot between $SLD = 100\%$ and $SLD = 0.1\%$ was taken to be an index of storage stability (SSI). One can observe from Figure 1 that a powder of greater stability will take longer to reach $SLD = 0.1\%$ and result in a greater area. In the nine storage tests $SLD = 0.1\%$ was reached in from 1 to 12 wk., depending on the rate of formation of off-flavors.

SSI was plotted against the reciprocal of initial HMF for nine stored controls (Figure 2). An analysis of variance showed this relationship to be significant ($p \cong 0.025$).

Drying temperature. Twenty-five drying runs were made for the temperature study. The commonly used method for expressing the influence of temperature on reaction velocity is that of Arrhenius.[2] The usual plot is the log k vs. $1/T$. If we may describe the reaction by an irreversible rate equation $r = k\ (C_r - C_p)^n$,[3] an approximately linear relationship between k and r is to be expected when C_r is much greater than C_p. This assumption is justified because the concentration of lactose or casein is of the order of 10^4 to 10^6 times the concentration of the product (HMF). Thus, the data can be treated by plotting log r vs. $1/T_m$.

Five of the 25 runs yield zero or negative rates of formation of HMF, where $r = \text{rate} = \dfrac{[HMF\ (\text{dried powder}) - HMF\ (\text{feed milk})].}{\text{Drying time}}$ Accordingly, the remaining 20 points were plotted as above (Figure 3). This relationship was analyzed statistically by an analysis of variance and found very highly significant ($p < 0.001$). The regression coefficient was −4.398 and it was assumed that the slope corresponded to E/R as defined by the Arrhenius equation. In

[2] The Arrhenius equation is frequently shown as:

$$k = Ae^{\frac{-E}{RT}}$$

where;

k = rate constant
A = constant, referred to as frequency factor
E = Activation energy
R = Gas constant
T = Temperature, absolute

See, for instance, Smith's Chemical Engineering Kinetics, McGraw-Hill. 1956.

[3] C_r = conc. reactant at zero time
C_p = instantaneous conc. product formed
n = 1, 2, . .

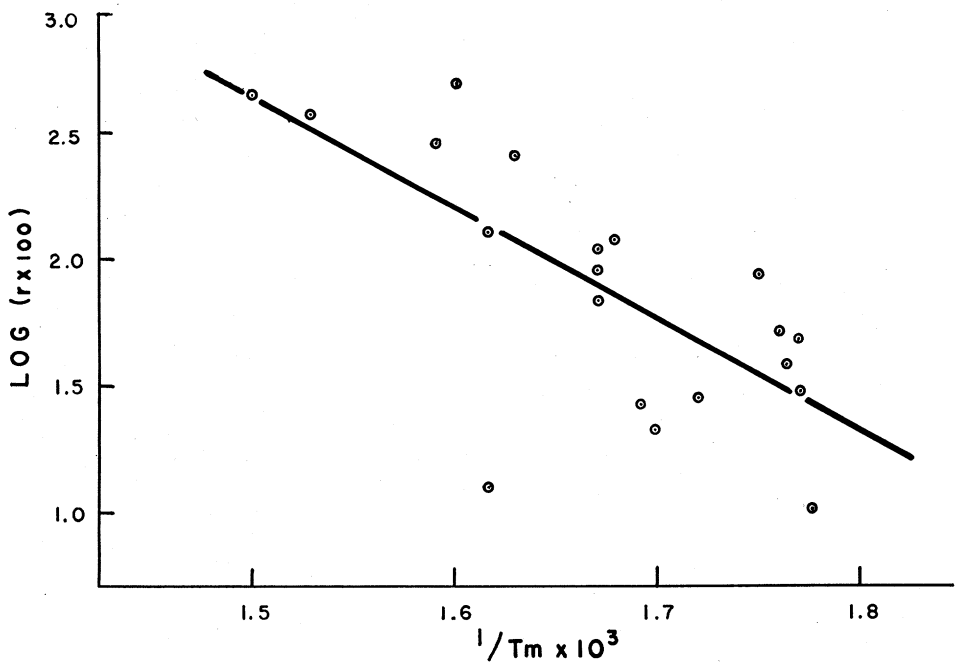

FIG. 3. Log ($r \times 100$) (rate of formation of HMF during drying) vs. $\frac{1}{T_m}$ maximum foam temperature. r is expressed in μM/liter-hour, and T_m in °R.

order to consider all of the drying runs, this value was substituted and the twenty-five points plotted as r vs. $e^{\frac{-E}{RT_m}}$ (Figure 4). This relationship was also analyzed statistically and found much more significant than that in Figure 3.

DISCUSSION

The RPC test is quite sensitive, but nonselective with respect to off-flavors. One should note that a powder significantly less fresh than the fresh control at the 0.1% level is not necessarily unacceptable. However, this is a good means of detecting early storage off-flavors in the powder.

Statistical analysis of duplicates of HMF run on these powders showed an error of ± 0.8 μM/liter at the 95% confidence level. With HMF values of about 1-5 μM/liter, this provides a fair amount of scatter, and is the most plausible reason for the zero and negative rates discussed earlier, as these values fell within experimental error.

The plot, Storage Stability Index (SSI) vs. $\frac{1}{\text{HMF}}$ (Figure 2) is sufficient to show qualitatively the relationship between storage stability and the initial HMF of the product within the range of 1 to 5 μM/liter. Thus, the lower the HMF content the more stable the product.

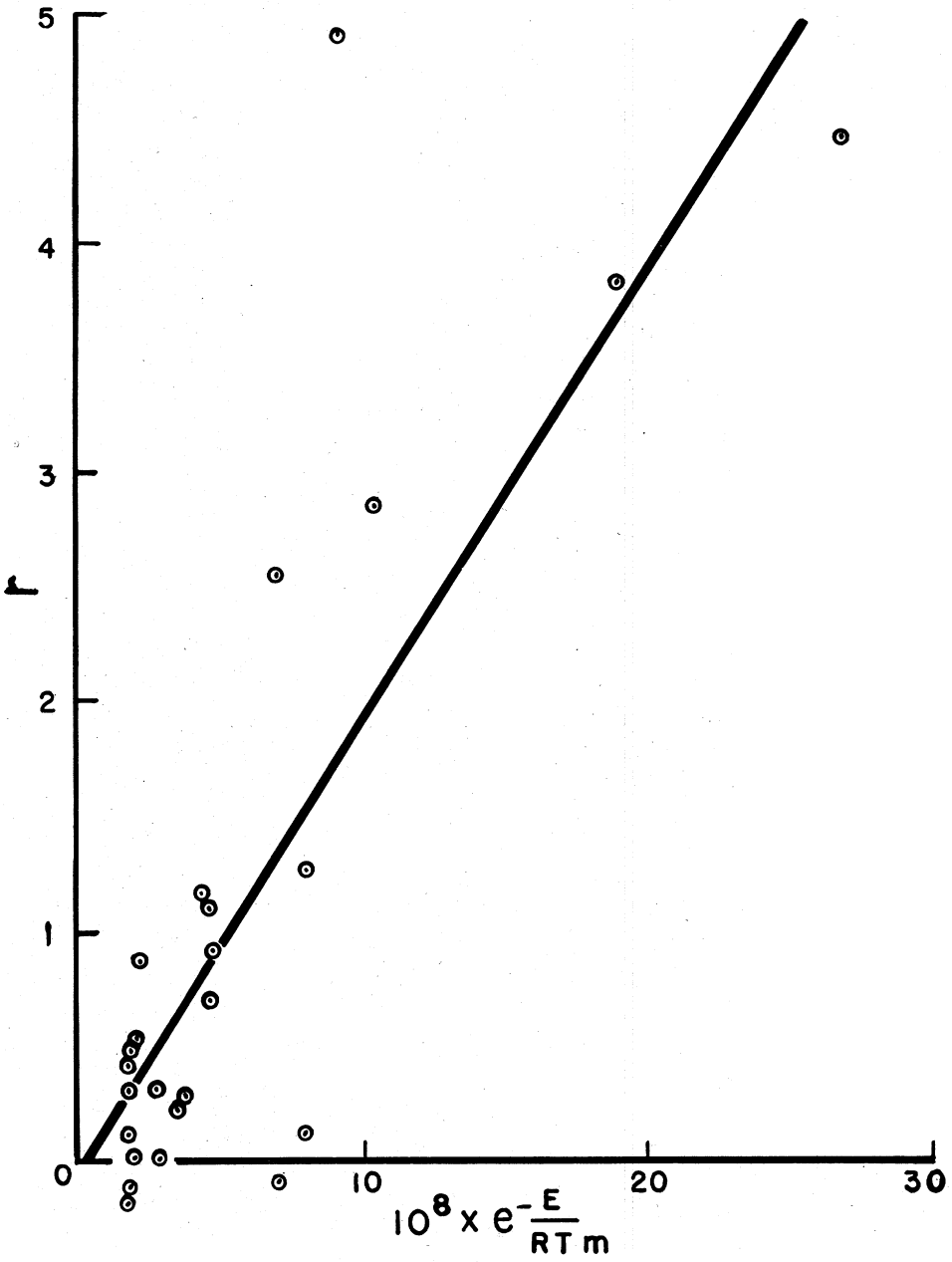

FIG. 4. r (rate of formation of HMF during drying) vs. $e^{\frac{-E}{RT_m}} \times 10^8$. T_m is maximum foam temperature and E/R was calculated from slope of plot in Figure 3.

This may indicate that the HMF analysis measures the potential for production of browning off-flavors on storage or HMF may simply be an index of heat

exposure during processing and thus indirectly indicates the initiation of nonbrowning reactions which produce off-flavors.

From Figures 3 and 4, HMF has been shown to be a good index of heat exposure and, given an HMF change during drying, one may estimate the time-temperature relationship.

As further substantiation of the assumptions, one may note that, although the expected point (0, 0) was not considered in the statistical analysis of Figure 4, extrapolation of the derived relationship to $T = 0$ gives virtually a zero rate of formation.

An activation energy, E, could be calculated from Figure 3, but may be of little meaning because it would be for varying amounts of products of three consecutive reactions. Therefore, it is not presented.

Drying times used in the calculation of rates were from time of application of heat to the foam to time of cooling the dried foam before removal. This is the more convenient variable, but some variation will exist between this and time-at-T_m.

A firmer basis for relating rate of formation of HMF to drying temperature would be to use the local temperature in the foam, or an average temperature integrated over the foam volume and time. However, either of these would be quite difficult to observe or estimate and would be of less practical value as a control parameter. This does indicate that a variable relationship between T_m and T (local) should contribute to a greater dispersion on the plot.

An additional variation should be due to change in HMF during the evaporation of the fluid milk. This, however, is done at 88-90° F. and fairly short times, so that variation is assumed small.

CONCLUSIONS

An estimate of the flavor stability of a number of foam-dried whole milk powders could be made from the HMF analysis at zero time.

A relationship was found between rate of formation of HMF and maximum foam temperature during drying, showing this analysis to be a good index of heat exposure. This should aid in establishing process conditions which will maximize flavor stability in the whole milk powder.

ACKNOWLEDGMENT

The authors express their thanks to F. B. Talley for conducting the organoleptic evaluations, to M. C. Lawson for conducting numerous HMF analyses, and to T. F. Holden for some of the early temperature studies.

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